AMENDMENTS TO THE SPECIFICATION

In the Specification:

Please amend the specification as shown:

Please delete the paragraph on page 4, lines 1-12 and replace it with the following paragraph:

-- Using the internal peptide sequence, Seq ID No. [[6]] **8** (VKPTSVPAFRPGAQFK) a 100% identity was obtained with 16 amino acids of the cosmid CY349. The corresponding gene was later annotated and designated as the *hupB* gene (Rv 2986c, Cole et al., 1998). The protein was found to be localized in the cytoplasm and on the cytoplasmic surface of the mycobacterial membrane, by immuno-gold electron microscopy. The *hupB* gene has been classified among the DNA binding (histone like) proteins of *M. tuberculosis* (Cole et al., 1998). Primers were designed to amplify the *hupB* gene. A 645 bp amplicon was obtained in case of *M. tuberculosis*. The α^{32}P labeled PCR amplicon was used in Southern hybridization to establish the size, prevalence and organization of the *hupB* gene in members of the MTB complex (*M. tuberculosis* and *M. bovis*) and other mycobacterial species. --

Please delete the paragraph on page 8, lines 11-22 and replace it with the following paragraph:

-- **Panel B, C and D :** The ethidium bromide stained amplification fragments of *M. tuberculosis* and *M. bovis* generated using primer pairs **N** (Seq ID No. 1) & **S** (Seq ID No. 2) (Panel **B**), **M** (Seq ID No. 3) & **S** (Seq ID No. 2) (Panel **C**) and **F**(Seq ID No. 4) & **R** (Seq ID No. [[1]] **5**) (Panel **D**) were electrophoresed on polyacrylamide gels. The 645 and 618 bp (Panel **B**); 318 and 291 bp (Panel **C**); 116 and 89 bp (Panel **D**); fragments have been indicated . Lanes 1 & 4, 645 bp, 6 & 10, 318 bp , and 13, 116 bp of the of *hupB* gene / C terminal part of the gene amplification fragment obtained in *M. tuberculosis* H37Rv ; lanes 2 & 5, 618 bp of *hupB* gene, 7 & 9, 291 bp and 11,12,15-17, 89 bp of the *hupB* gene / C terminal part of the gene amplification fragment obtained in *M bovis* AN5 ; 3, 8 & 14, 100 bp molecular weight markers. --

Please delete the paragraph on page 11, lines 5-14 and replace it with the following paragraph:

-- **Fig: 5 Nucleotide sequence alignment of *hupB* gene of *M. tuberculosis* and *M. bovis*:**

The nucleotide sequence of the C-terminal region (326-676 bp) of *hupB* gene of standard strains of *M. tuberculosis* and *M. bovis* and clinical isolates of *M. bovis* has been aligned using GCG software. A deletion of 27 bp was seen in *hupB* sequence of all *M. bovis* strains. The 9 deleted amino acids (KAATKAPAR) **(SEQ ID NO: 11)** between 385 to 411bp with respect to *M. tuberculosis* are shown in single letter code on the first line. Numbers in brackets refer to nucleotide position in *hupB* (*Rv2986c*). The *M.bovis* strain numbers are given on the left. **Figure 5 discloses SEQ ID NOS 11, 12, 12, 13, 13, 13, 13, 13, 13, 13, 13, 14 and 13, respectively, in order of appearance.** --

Please delete the paragraph on page 13, lines 11-13 and replace it with the following paragraph:

-- Another embodiment is a method wherein the DNA probe consists of sequence ID No. [[7]] **6** or sequence ID No. [[8]] **7** or a complement thereof tagged with a detectable label. --

Please delete the paragraph on page 15, lines 7-8 to and replace it with the following paragraph:

-- Another embodiment is *Hup B* gene (Seq ID No. [[8]] **6**) substantially as herein described a process as in preceding embodiments has been substantially described. --

Please delete the paragraph on page 17, lines 10-16 and replace it with the following paragraph:

-- **Nested PCR:** A method for differentiating *M. tuberculosis* and *M. bovis*. Comprising the steps of amplifying a part of the target *hup B* gene from *M. tuberculosis* and *M. bovis* in a polymerase chain reaction. The PCR fragment obtained with primers **N,** Seq. ID No.1[[-N]] and **S,** Seq. ID No.2[[-S]] was used as target DNA in nested PCR. The C- terminal portion of the gene was also amplified by using **F,** Seq.ID. No.4[[-F]] and **R,** Seq.ID. No.5[[-R]] the expected amplicon was ~ 116 bp in case of *M.tuberculosis* and 89 bp in case of *M.bovis*,

(ii) using primers **F, Seq ID No. 4** (5' ccaagaaggcgacaaagg3') with **R, , Seq ID No.** 5 (5' gacagctttcttggcgggg3'). The expected size of the amplicon was 116 bp in case of *M. tuberculosis*, and 89 bp in case of *M. bovis* respectively. --

Please delete the paragraph on page 19, lines 12-19 and replace it with the following paragraph:

-- Results, obtained with the amplicon generated in the C - terminal portion of the gene using **M** and **S** primers on digestion with *Hpa*II, showed differences matching to the differences seen in case of the PCR fragment obtained using the *hupB* primers (**N, Seq ID No. 1[[- N]]** and **S, Seq ID No. 2[[- S]]**) indicating that the PCR-RFLP assay utilizing either the PCR fragment obtained using the *hupB* primers (**N, Seq ID No. 1[[- N]]** and **S, Seq ID No. 2[[- S]]**) / the C terminal primers (**M, Seq ID No. 3[[- M]]** and **S, Seq ID No. 2[[- S]]**) did distinguish between *M. tuberculosis* and *M. bovis*. --

Please delete the paragraphs on page 22, line 5 to page 23, line 3 and replace them with the following paragraphs:

-- **Nested PCR**: DNA extracted from clinical samples / cultivated mycobacteria were processed for PCR with primers **N,** Seq. ID No.1[[-N]] and **S,** Seq. ID No.2[[-S]] . The PCR product obtained using the primers **N,** Seq.ID. No.1[[-N]] and **S,** Seq.ID. No.2[[-S]] was used as target DNA in nested PCR. --

-- Each reaction (40μl) contained 2.5 mM $MgCl_2$, 0.5 μM of primers, 200 μM dNTPs, 10 mM Tris-HCl (pH 8.8 at 25°C), 50 mM KCl, 0.08% Nonidet P40, and 0.5 Units of Taq DNA Polymerase. The PCR reaction was subjected to initial denaturation at 95°C for 10 min., and 35 cycles of 1 min at 94°C, 1 min., and 30 seconds at 59°C and final extension at 72°C for 7 mins. The fragments were analyzed on a 3.5 % agarose gel / 8 % non-reducing polyacrylamide gel and stained with ethidium bromide. The C- terminal portion of the gene was also amplified by using **F,** Seq.ID. No.4[[-F]] (5' ccaagaaggcgacaaagg3') with **R,** Seq.ID. No.5[[-R]] (5' gacagctttcttggcgggg3'), the expected amplicon was ~ 116 bp in case of *M.tuberculosis* and 89 bp

in case of *M.bovis*, (Table II, Fig:1). --

-- **Southern Hybridization**: The PCR amplicons resolved on the agarose gel were transferred on to nitro-cellulose membrane (Southern, 1975). The blots were then hybridized with α-^{32}P labeled 645 bp *hupB* (Seq ID No.6) gene probe from *M. tuberculosis*, (*Pst*I & *Nco*I digest from the plasmid pHLPMT / probe generated by PCR using **N** (Seq. ID No.1[[-N]]) and **S** (Seq. ID No.2[[-S]]) primers and *M. tuberculosis*, DNA.). --

Please delete the paragraphs on page 15, line 21 to page 26, line 14 and replace them with the following paragraphs:

-- **Sensitivity of *hupB* gene based PCR assay**: The sensitivity of DNA PCR amplification (level of detection) was established by adding serial dilutions of mycobacterial DNA (1 ng to 1 fg) in the PCR reaction using primers **N, Seq ID No. 1[[- N]]** and **S, Seq ID No. 2[[- S]]**. It was seen that by ethidium bromide staining alone the detection limit was 50 pg and by hybridization the detection limit increased to 500 fg (Fig: 3A and B). This was equivalent to the detection of 5000 and 50 genome equivalents respectively. --

-- **RFLP of PCR Amplicons of *hupB* gene derived from *M. tuberculosis* and *M. bovis***: DNA from different isolates of *M. tuberculosis* and *M. bovis* (listed in Table I) were amplified using **N, Seq ID No. 1[[- N]]** and **S, Seq ID No. 2[[- S]]**primers (645 bp fragment, Table II) and (ii) **M, Seq ID No. [[3- M]]** (internal primer) and **S, Seq ID No. 2[[- S]]** (318 bp fragment, Fig: 4C, Table II, Fig: 1). PCR amplicons obtained from the DNA of *M. bovis* strains (lanes 4-11, Fig: 4B and 4C) were smaller in size as compared to the PCR amplicons obtained from the *M. tuberculosis* strains (lanes 1-3, Fig:4B and 4C). The results of the PCR assay with the 2 sets of primers have been summarized in Table III. --

-- In order to confirm the difference in 645 and 618 bp PCR fragment sizes, the amplicons were digested with *Hpall* and *Haelll* (Fig.:4D). The digested fragments were analyzed on 12% non-denaturation polyacrylamide gel. Digestion of 645 bp fragment with *Hpall* clearly revealed that

in case of *M. bovis* a ~250 bp (Fig: 4D, lane 3) fragment obtained was smaller in size compared to the ~280 bp bands obtained with *M. tuberculosis* H37Ra & H37Rv (Fig: 4D, lanes 1 and 2). No differences were perceived with *Haelll* digestion, (Fig: 4D, lanes 5-8). Results, obtained with the amplicon (318 bp) generated in the C – terminal portion of the gene using **M, Seq ID No. 3[[- M]]** and **S, Seq ID No. 2[[- S]]** primers on digestion with *Hpall*, showed similar differences (results not shown) indicating that the PCR-RFLP assay did distinguish between *M. tuberculosis* and *M. bovis* strains. --

-- **Sequencing of PCR Amplified Fragment**: PCR amplicons obtained from DNA of standard strains of *M. bovis* and *M. tuberculosis* including local isolates of *M. bovis* derived from cattle were sequenced. The PCR amplicons 618 and 645 bp (obtained using **N,** Seq. ID No.1[[-N]] and **S,** 2[[-S]]), 318 and 291 bp (obtained using **M,** Seq ID No. 3[[-M]] and **S,** 2[[-S]]), 116 and 89 bp (obtained using **F,** Seq ID No. 4[[-F]] and **R,** 5[[-R]]) were sequenced to confirm the size differences. Sequence analysis indicated that in *M. bovis* there was a deletion of 27 bp (9 amino acids) in frame after 128th codon in the C terminal part of the gene (Fig: 5). The histone like gene sequence of *M. bovis* (Accession No.Y18421) and *M. tuberculosis* (Accession No. P95109) has been submitted to the NCBI database. --

Please delete the Table II and replace with the following table:

Table II: Primers Used for Amplification of *hupB* Mycobacterial DNA Target

Primer Pair	Sequence of Primer	Target *hup B* gene	Mycobacteria	PCR Product Size
N, Seq.Id. No.1[[-N]]	(5'ggagggttgggatgaacaaagcag 3')	Whole gene	*M. tuberculosis*	645 bp
S, Seq.Id. No.2[[-S]]	(5' gtatccgtgtgtcttgacctatttg 3')		*M. bovis*	618 bp
M, Seq.Id. No.3[[-M]]	(5' gcagccaagaaggtagcgaa 3')	C terminal	*M. tuberculosis*	318 bp
S, Seq.Id. No.2[[-S]]	(5' gtatccgtgtgtcttgacctatttg 3'),		*M. bovis*	291 bp
F, Seq.Id. No.4[[-F]]	(5' ccaagaaggcgacaaagg3')	C terminal	*M. tuberculosis*	116 bp
R, Seq.Id. No.5- **R**	(5' gacagctttcttggcggg3').		*M. bovis*	89 bp

Please delete page 33 and replace it with the following page:

SequenceName : **seq id no. 6 [[seq id no. 8]]**

OrganismName : hup B - *M. tuberculosis*, Rv2986c, Accession No. P95109

atgaacaaag cagagctcat tgacgtgctc acacagaaat tgggctcgga ccgtcggcag 60
gcgaccgccg ccgtcgagaa tgtcgttgac acgattgtgc gtgcggtaca caaaggcgac 120
agcgtcacca ttaccgggtt cggtgtgttc gaacagcgtc gccgcgcggc tcgagtggcc 180
cgcaatccgc gtaccggcga gacagtaaag gtgaagccga cgtcggtgcc ggcgttccgc 240
ccgggcgcgc aattcaaagc ggttgtgtct ggcgcgcagc gtctcccggc agaaggaccc 300
gctgttaagc gtggtgtggg ggccagtgca gccaagaagg tagcgaagaa ggcacctgcc 360
aagaaggcga caaaggccgc caagaaggcg gcgaccaagg cgcccgccag gaaggcggcg 420
accaaggcgc ccgccaagaa agcggcgacc aaggcgcccg ccaagaaagc tgtcaaggcc 480
acgaagtcac ccgccaagaa ggtgaccaag gcggtgaaga agaccgcggt caaggcatcg 540
gtgcgtaagg cggcgaccaa ggcgccggca aagaaggcag cggccaagcg gccggctacc 600
aaggctcccg ccaagaaggc aaccgctcgg cggggtcgca aatag 645

SequenceName : **Seq id no.7 [[seq id no. 6]]**

OrganismName : Hlp of *Mycobacterium bovis*, Accession No. Y18421

atgaacaaag cagagctcat tgacgtgctc acacagaaat tgggctcgga ccgtcggcag 60
gcgaccgccg ccgtcgagaa tgtcgttgac acgattgtgc gtgcggtaca caaaggcgac 120
agcgtcacca ttaccgggtt cggtgtgttc gaacagcgtc gccgcgcggc tcgagtggcc 180
cgcaatccgc gtaccggcga gacagtaaag gtgaagccga cgtcggtgcc ggcgttccgc 240
ccgggcgcgc aattcaaagc ggttgtgtct ggcgcgcagc gtctcccggc agaaggaccc 300
gctgttaagc gtggtgtggg ggccagtgca gccaagaagg tagcgaagaa ggcacctgcc 360
aagaaggcga caaaggccgc caagaaggcg gcgaccaagg cgcccgccaa gaaagcggcg 420
accaaggcgc ccgccaagaa agctgtcaag gccacgaagt cacccgccaa gaaggtgacc 480
aaggcggtga agaagaccgc ggtcaaggca tcggtgcgta aggcggcgac caaggcgccg 540
gcaaagaagg cagcggccaa gcggccggct accaaggctc ccgccaagaa ggcaaccgct 600
cggcggggtc gcaaatag 618